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BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

JULY, 1942

THE ANTI-COAGULANTS

Heparin and the Dicoumarin-3, 3′ Methylene-Bis-(4-Hydroxycoumarin)*

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THE problem of inhibition of coagulation has been the subject of numerous and frequently conflicting reports during the past two decades. From this material pertinent findings will be briefly reviewed in order to present a background for the consideration of Heparin and the Dicoumarin-3, 3′ Methylene-Bis-(4-Hydroxycoumarin). The conclusions suggested should probably be considered as representing current opinion rather than final truth.

Prothrombin, a protein normally present in the blood plasma in a concentration of approximately 40 mg. per cent,[1] is quantitatively converted into thrombin by the action of thrombokinase and ionic calcium.

Prothrombin is the component chiefly affected by 3, 3′ methylene-bis-(4-hydroxycoumarin),[2] a clotting inhibitor which will be discussed at length later. We shall, therefore, dwell upon it briefly. The liver is

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the chief source of prothrombin.[3, 4, 5, 6] Vitamin K appears to be important in its formation.

Hypoprothrombinemia may occur in conditions associated with insufficient vitamin K intake, absorption and utilization. Reduction in prothrombin concentration to below 35 per cent of normal, will, in many instances, produce a hemorrhagic state. Avitaminosis K on the basis of dietary insufficiency rarely occurs, possibly due to the capacity of the intestinal flora to synthesize the vitamin when such a deficiency exists.[7] Absorption of vitamin K is contingent upon an adequate concentration of bile salts in the gastrointestinal tract,[8, 9] hence obstructive jaundice and biliary fistulae are frequent causes of hypoprothrombinemia. Diminished prothrombin concentration occurs in ulcerative colitis, sprue, intestinal obstruction and polyposis,[10, 11, 12, 13] due to interference with absorption of vitamin K through the intestinal wall.

Prothrombin deficit despite adequate vitamin K intake and absorption may occur in hepatic derangements.[13, 14, 15, 16, 17, 18] Hepatic intoxications (due to carbon tetrachloride,[19] chloroform,[3] and phosphorus), hepatectomy[5] and extensive liver damage on other bases[13, 17, 18] result in a hypoprothrombinemia in no way influenced by the administration of large doses of vitamin K. DeLor and Reinhart[20] observed prothrombin to be universally diminished when liver function fell below 50 per cent, as determined by the hippuric acid method.

Calcium, as ionic calcium, hastens the conversion of prothrombin to thrombin.[21] For calcium deficiency to significantly inhibit coagulation, however, serum calcium would have to fall below the lowest recorded level compatible with life.[22]

Thrombokinase is a lipoprotein closely allied to cephalin. In combination with calcium it is considered by Eagle[23] to constitute a proteolytic enzyme analogous to trypsin, which reacts with prothrombin to form thrombin. Crude or crystalline trypsin[24] and proteolytic snake venoms[25] can be substituted for calcium-platelet or calcium-tissue-extract mixture in the activation of prothrombin in vitro. The view that activation of prothrombin involves proteolysis would seem to be supported by these reactions. Thrombokinase is present in all tissues; the lung, platelets, and brain being its richest sources. Thrombokinase affects only the velocity of the prothrombin to thrombin conversion not the quantity of the thrombin derived from prothrombin.[26, 42] Heparin antagonizes thrombokinase,[27] blood remaining fluid despite the presence

of large quantities of circulating prothrombin and fibrinogen, only as long as the thrombokinase-heparin balance is maintained in favor of heparin, and in all probability certain other anti-coagulants about which little is known.

Thrombin, a protein, is probably a hydrolytic product of prothrombin. Schmidt[28,29] and Eagle[30] believe it to be a proteolytic enzyme which converts fibrinogen into fibrin. Thrombin is capable of converting 2000 times its weight of fibrinogen into fibrin.[30] It may be replaced in the conversion of fibrinogen to fibrin by proteolytic enzymes such as papain[24] and numerous snake venoms (Crotalus adamantus, Crotalus terrificus, Crotalus horridus, Bothrops nummifera, etc.).[25] No apparent stoichiometric relationship exists between thrombin and fibrinogen.

Fibrinogen is a globulin-type protein of special solubilities. It is produced exclusively in the liver. Except in very severe forms of liver disease, the fibrinogen concentration in the blood is maintained at a normal level of 0.2 to 0.3 mg. per cent. Its conversion from the physical state of a disperse hydrosol to a quasi-crystalline fibrin gel is the *sine qua non* of natural blood clotting.

In vivo, anti-coagulants may be arbitrarily grouped in five classes:

1. Substances in nature which have anti-coagulant activity when added to blood; for example, hirudin, an anti-thrombin[31,32] and anti-kinase,[33] derived from the buccal gland of the leech, and snake venoms of the cobra venom type.

2. Anti-coagulant substances isolated from blood or tissue, e.g., heparin.

3. The physiological anti-coagulant presumed to be present because of delayed coagulation of the blood. This is believed by some to be heparin.[34] Others contend that it is an anti-thrombin distinct from heparin.

4. Substances which, when ingested, either depress formation of one of the components of the coagulation mechanism or render it inactive, e.g., dicoumarin.

5. Synthetic substances, which, when added to blood, inhibit coagulation; e.g., the diazo dyes (Chicago blue, chlorazol fast pink, sodium thiosulfate, trypan blue), germanin and liquoid.[35,36,37,41,50]

Since it is beyond the scope of this paper to discuss in detail the mechanism of, and the clinical results obtained with each of these substances, the major portion of the discussion will be devoted to heparin,

and dicoumarin, a recently isolated clot inhibitor which has been the subject of investigation in the Department of Medicine of the New York Post-Graduate Medical School and Hospital during the past year.

The limitations of hirudin which render its clinical use impracticable are its low potency, as compared to heparin, and its limited supply.

The synthetic in vivo anti-coagulants are similar to heparin in their mode of action.[38] With the exception of chlorazol fast pink, a very narrow margin exists between the therapeutically effective and the toxic dose. The anti-coagulant activity of chlorazol fast pink has been found to be 10 to 15 times less than that of heparin.

Heparin

Heparin is a strongly acidic compound containing mucoitin polysulfuric acid, acetic and glycuronic acid plus a base glucosamine.[39] It inhibits the coagulation of blood in vitro or in vivo by retarding the conversion of prothrombin to thrombin, and by direct antithrombic activity.[42] Heparin arises in the mast cells of Ehrlich[40] which are found chiefly in the vicinity of the finer blood vessels.[40] Jorpes[34] concluded from the location of the mast cells that they constitute a hormonal system feeding heparin to the blood. The liver capsule and the lung are the richest reservoirs of heparin in the body. Appreciable amounts have also been extracted from the subcutaneous tissue and the blood vessels. Histologically, heparin inclusions in the mast cells may be detected by the characteristic metachromatic stain which they yield with toluidine blue.

Ferguson[42] has demonstrated that heparin possesses two distinct actions in the first phase of the clotting mechanism. First, it retards the rate of prothrombin conversion to an extent which is inversely proportional to the amount of the thrombokinase present. Second, there is an effect on the amount (effectiveness) of the thrombin formed.[43] Heparin apparently not only inhibits coagulation by retarding the formation of thrombin, but also by acting as an anti-thrombin.[22,44,45] Quick,[46] Brinkhous,[47] Jaques and Mustard[48] have demonstrated that heparin is only active in the presence of a co-factor which is part of the albumin fraction. In their opinion, heparin combines with and enhances the action of normal plasma anti-thrombin. Trypsin[49] and thrombokinase[27] directly antagonize heparin, coagulation being prevented unless an excess of these substances is present. Many clot inhibitors resemble heparin

in their mode of action and in the presence of sulfur in the molecules. For example, sulfo-cellulose, hermophenyl, liquoid, germanin, diazo dyes, cystine, and sodium thiosulfate, like heparin, exhibit an anti-coagulative activity proportional to their sulfur content.[38,39,40,50] Lindgren and Wilander[51] and Hedenius[52] observed that with heparin the coagulation time may be maintained at optimal levels of twenty to thirty minutes without influencing the bleeding time as determined by the Ivy method. Best[53] demonstrated, by means of a glass cell technique, that platelet agglutination, as well as fibrin deposition, was inhibited by heparinization. Subsequently, Solandt and Best[54] presented evidence that very extensive injury to the arteries and veins never resulted in a maximal stimulus to platelet agglutination. The amount of heparin required to prevent platelet thrombi in vivo was found to be much smaller than that required to prevent the process in a glass cell. Salmine-sulfuric acid, a protamine, neutralizes the effect of twice the amount of heparin in vitro and in vivo. An intravenous injection of this protamine will immediately check any undesirable bleeding produced by heparin.[55,56]

Preparations: The products available for clinical use are:

1. Heparin—Lederle 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.
2. Liquemin—Roche Organon 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.
3. Solution of heparin—Connaught Laboratories 10 cc. vial—10 mg./cc.-1100 Toronto u/cc.

These products are at present so standardized that each cc. keeps 5000 cc. of plasma in vitro in a liquid state for four hours at 37° C.

Methods: The methods of heparinization in use today are local, regional and general. Local and regional heparinization are used chiefly in vascular surgery in order to confine the anti-coagulant effect to the operative area. In the treatment of thrombosis and thrombo-embolic conditions, general heparinization is the method of choice. This may be achieved by either of two techniques: (1) By the continuous intravenous drip; and (2) by multiple intravenous injection. In the first, advocated by Best and Murray and utilized widely in this country, the appropriate amount of heparin is added to an infusion which is permitted to flow continuously throughout the course of therapy into the selected vein. The choice of diluent may be modified by the individual case, heparin being equally active in five per cent glucose solution,

normal saline, or Ringer's solution. Should fluid restriction be imperative, the entire daily dose may be given in as little as 800 to 1000 cc. The amount of heparin required to secure an arbitrary optimal prolongation of coagulation time, namely, twenty to thirty minutes, varies according to the response of the individual patient. Twenty to thirty mg. per hour usually suffice to maintain this level. Variation in response in different patients and in the same patients from day to day will of necessity modify the dose. In our opinion coagulation times in excess of forty minutes are hazardous. Maintenance of a coagulation time of twenty to thirty minutes is insured by repeated estimations of the venous blood clotting time at four hour intervals by means of the Lee-White two tube method. The intermittent intravenous injection method has been used almost exclusively by Crafoord,[57] Jorpes,[34] and Lindgren and Wilander[51] since 1936. These observers have been using doses of from 50 to 75 mg. three times daily at 8 A.M., noon and 4 P.M. with an evening dose of 100 to 125 mg. at 8 P.M. Appropriate adjustments in the dosage are made as indicated by the coagulation time obtained. The marked fluctuations in coagulation time encountered with this method render dosage estimation problematical. Dosage should theoretically be so regulated that the coagulation time never falls below 15 minutes, but this is very difficult to achieve with this technique. The disadvantage of multiple venipuncture may be obviated by employing a specially constructed needle designed by Olovson.[58] This needle is allowed to remain in situ throughout the course of therapy. It is supported by an aliform plate which is fixed to the arm by adhesive tape and is provided with a detachable cap with a rubber membrane through which the injections may be made even while the patient is sleeping. Coagulation has never been observed in the needle during the course of treatment.[59]

Certain theoretical disadvantages are inherent in this method of heparin administration. Chief among these is the impossibility of maintaining the more or less constant elevation of coagulation time secured with the continuous intravenous drip. Secondly, the coagulation time immediately following injection of undiluted heparin attains levels, which may be hazardous, of one hour or more. Subsequent to the initial abrupt rise, there is a steady fall over a four hour interval to as low as eight to ten minutes in some cases. The foregoing drawbacks notwithstanding, these authors have reported satisfactory results with the intermittent intravenous injection technique.

Heparin has also been administered by the subcutaneous route. The magnitude of the doses required to obtain the desired levels with this method renders it impracticable.

Because of the voluminous available literature concerning the indications for the therapeutic and prophylactic use of heparin, we shall confine ourselves to a brief summary of recent clinical and experimental developments. Regional and general heparinization have proved invaluable in maintaining vessel patency following arteriotomy for the removal of thrombi and emboli. Murray,[60] Lam,[61] Lindgren and Wilander[51] have reported successful embolectomies with this technique. Pratt, of our service, has obtained similar results with heparin in six embolectomies and in twelve other instances of vascular surgery, hitherto usually unsuccessful because of the rapidity with which the repaired vessel became obstructed by thrombi, during the postoperative period. In mesenteric thrombosis, with a mortality rate of from 85 to 95 per cent, heparin was employed successfully by Murray[60,62] in six cases. Ravdin[63] reported comparable results in two instances. Holmin and Ploman[64] demonstrated that heparinization of patients with thrombosis of the central retinal vein appreciably reduced the incidence of blindness following this disease. This observation has been confirmed by Boström and Olsson,[65] Rea[66] and other ophthalmologists. Whipple[67] and Murray[68] observed that postoperative heparinization will prevent the portal thrombosis which frequently follows splenectomy for Banti's syndrome and familial jaundice. Recovery in patients with massive pulmonary embolization appears to be influenced by heparin therapy. The authors have successfully treated five such patients in whom the initial prognosis was extremely grave. Murray,[68] Ravdin,[63] Clason,[69] Rosenqvist[59] and Priestley[70] have reported similar experiences, observing likewise a marked diminution in morbidity as well as mortality. The fact that spontaneous recovery does occur in approximately 12.2 per cent of cases of pulmonary embolization, according to Barker's[71] statistics, renders observation of a large series desirable before any conclusion can be drawn as to the efficacy of this form of therapy. The treatment of subacute bacterial endocarditis with heparin and sulfonamide derivatives has yielded encouraging results in a few of the cases reported by Kelson and White.[72] In the case reported by Dockeray and Kawerau,[73] which terminated in a fatal cerebral hemorrhage, heparin was only of transient value. Other reports[74,75,76,77] which ap-

pear in the literature reveal that fatal cerebral hemorrhage following embolization not infrequently occurs in patients receiving combined heparin and sulfanilamide therapy for subacute bacterial endocarditis. The value of this combined therapy is yet to be determined. It has failed in nine cases on our service.

The value of heparin therapy in coronary thrombosis is still speculative. Solandt and Best[78] showed that preliminary heparinization will prevent thrombosis of coronary arteries injected with sodium ricinoleate. This work seems to indicate that if administered early, and in adequate dosage, heparin may minimize or inhibit propagation of the thrombus. At present, however, the prevention of mural thrombi seems to be its sole virtue in this disease.

Lyons,[79] Schall,[80] Ershler and Blaisdell[81] have reported the successful treatment of cavernous sinus thrombosis by combining heparin with chemotherapy.

Marked improvement in a single case of thrombosis of the posterior inferior cerebellar artery (Wallenberg syndrome) following heparinization has been reported by Magnusson.[82]

In a series of 627 patients reported by Crafoord,[57,83] 325 who had received heparin prophylactically during the postoperative period showed a complete absence of thrombo-embolic phenomena. The remaining 302 patients subjected to comparable operative procedures, who had not received the anti-coagulant, showed an incidence of thrombo-embolic complications of approximately 9 per cent. (This figure is considerably greater than that encountered in this country.) Nine of this latter group died. Diagnosis was confirmed by autopsy. Similar observations supporting the efficacy of heparin as a prophylactic against thrombosis following obstetric and gynecologic procedures have been reported by Wetterdal,[84] Leissner[85] and others.

The numerous variations in the clinical picture accompanying thrombophlebitis, and the frequently insidious onset of this condition account in some measure for failure of its early detection. Most cases, if diagnosed in their incipiency, respond favorably to heparinization. Localization of the thrombus to its original site, accompanied by marked diminution in embolic complications, morbidity and mortality, are the advantages reported by numerous observers. On the other hand, the difficulty inherent in its use, together with other objections to be discussed later, have led many groups to confine its use

in thrombophlebitis to those patients who have suffered one pulmonary embolus. Venography in suspected cases, according to Bauer's[86] recent report, gives promise of affording a means of earlier and more certain recognition of incipient thrombophlebitis.

An interesting recent development in heparin therapy, while not related to the cardiovascular system, is worthy of mention. This is the prevention of adhesions by instillation of heparin into the peritoneal cavity of rabbits subjected to contamination and trauma.[87] In dogs, the recurrence of divided adhesions was 6 times greater in the non-heparinized group as compared to that in heparinized animals. Wright and Hinton[88] have tried this in man with apparently good results. Definite conclusions as to the efficacy of intraperitoneal heparinization in man cannot yet be drawn inasmuch as no opportunity to reëxamine the abdominal cavity has presented itself. Some danger of hemorrhage following closure does exist, when the initial contraction of the small vessels wears off three to four hours postoperatively; hence routine use of heparin intraperitoneally is not to be recommended at this time.

There exists some difference of opinion as to how long heparin therapy should be continued. Following vascular surgical procedures in which the lumen is kept patent by heparinization, the site of arterial suture has been found experimentally[68] to be healed after 72 hours. Murray[68] and Lindgren[51] prefer to continue heparinization postoperatively until the patient is out of bed. In thrombophlebitis Bauer[86] discontinues therapy after five days in abortive cases; in certain instances he has withheld heparin as soon as the temperature and pulse returned to normal. Hedenius[52] proposed four days as the minimum effective period of therapy. In our experience such periods have frequently proven too short. In the majority of cases of thrombophlebitis the intravascular inflammatory process persists despite inhibition of thrombus propagation. We feel, therefore, that in order to minimize the tendency to relapse the administration of heparin in quantities adequate to maintain coagulation time at optimum levels should be continued over a period of from twelve to fourteen days. Withdrawal of heparin is followed by a so-called negative phase during which the blood exhibits increased coaguability. For this reason heparin should never be discontinued abruptly but rather by a successive slow diminution in dosage. On our service the following criteria have been adopted as evidence of cessation of the intravascular process in thrombophlebitis: Return of

temperature and pulse to normal for one week; complete disappearance of local tenderness, pain, and erythema along the course of the involved vessel over a similar period of time; the sedimentation rate and leukocyte count, if elevated, should likewise return to and maintain a normal level for one week; reduction of edema to a minimum is desirable. Strict adherence to the above routine has reduced, but not eliminated, the incidence of reactivation of the acute process after discontinuing heparin. As a rule, the briefer the period of heparinization the greater the possibility of recurrence. Reactivation of acute thrombophlebitis may definitely occur following heparinization. In an experience involving the heparinization of approximately 100 patients, the following examples have been encountered. Three patients in whom heparin therapy was maintained for two to five days following satisfactory clinical evidence of subsidence, showed reactivation shortly after cessation. Two others had recurrences after heparinization for ten days; two more showed reactivation of the process following fourteen days of heparin therapy, one of whom suffered a second recurrence following an additional fourteen days of treatment. We have also seen two instances of non-fatal pulmonary embolization while heparin therapy was maintaining the clotting time at levels of twenty-six to thirty minutes.

Complications: In 315 cases reported by Murray and Best[68] four developed hematomata of the wound. Priestley, Essex and Barker[70] observed transient hematuria in several of their forty-five patients. Ershler and Blaisdell[81] and Witts[76] reported the occurrence of massive hematuria following heparinization. Lam[61] in his series of thirty cases noted evidence of a hemorrhagic diathesis in four patients. Kelson,[72] Fletcher,[75] Friedman,[77] Witts[76] and Miller[74] have reported deaths as the result of cerebral hemorrhage following treatment of subacute bacterial endocarditis with heparin and sulfonamide drugs.

Although heparin is a valuable agent in anti-coagulant therapy, it does have certain disadvantages. Chief among these are:

1. Difficulty in administration with its attendant discomfort to the patient.
2. Costliness: Fifteen to twenty dollars worth of heparin is required daily in the average case.
3. Prolonged administration.
4. Occasional failures.

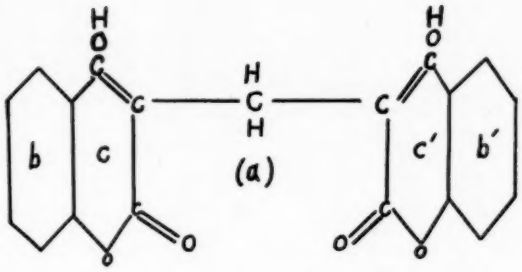

Fig. 1—Structural formula for dicoumarin 3, 3'-methylene-bis-(4-hydroxycoumarin).

Added to these are the dangers inherent in all types of anti-coagulant therapy, unless scrupulous control is observed.

Dicoumarin*

The isolation and synthesis of the dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin) by Link[89] and his co-workers in 1940 provided a new and valuable substance for workers interested in the field of anti-coagulants. Rather exhaustive studies in which this substance has been administered to rabbits, dogs, and other animals have been reported by Link[89] and his co-workers, and by Bingham, Meyer and Pohle.[106] As may be anticipated from a consideration of the structural formula shown in Figure 1, 150 substitution and degradation products of 3, 3′ methylene-bis-(4-hydroxycoumarin) have been isolated by Link's group,[90] more than 40 of which show anti-coagulant properties as measured by coagulation and prothrombin studies.

Dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin), when fed to susceptible animals, diminishes the clotting power of the blood and, in some cases, produces fatal hemorrhage. In nature, this compound occurs in spoiled sweet clover silage. It was for this reason that Roderick[96,97,98] and Schofield[94,95] used the name "sweet clover disease" to describe the hemorrhagic diathesis resulting in cattle from the ingestion of spoiled sweet clover hay. The coagulating power of the blood can be restored by discontinuing the administration of the hemorrhagic agent, or by injection of serum or whole blood freshly drawn from

* The Dicoumarin used in this study was supplied through the courtesy of Dr. Karl Paul Link, University of Wisconsin, and Dr. Y. Subbarow of the Lederle Laboratories.

cattle. Quick[99] confirmed Roderick's[97] observation that the coagulation defect in animals with sweet clover disease was due to a hypoprothrombinemia, the mechanism of which was, and still is, unknown.

Link[89,90,91,92,93] and his co-workers completed the isolation and synthesis of this dicoumarin in April 1940. The pharmacological activity of 3, 3′ methylene-bis-(4-hydroxycoumarin) has been established in animals by Link,[105] Bingham,[106] and Butt[107] with their associates. Link and his group have demonstrated that within certain limits, increasing the size of a single dose increases the degree and prolongs the time of reaction in standardized susceptible rabbits. These observers likewise demonstrated that dogs survived large single doses, whereas in some cases repeated divided doses aggregating less than the large single dose, produced toxicity and death. Link[109] suggests incomplete absorption from the gastrointestinal tract as an explanation of this variation in response.

Postmortem studies on animals that had succumbed to hemorrhage produced by the ingestion of spoiled sweet clover, were first made by Roderick[96] who noted that hemorrhage may occur in any part of the body, but most frequently at points of stress, in the subcutaneous and intermuscular fasciae. No changes in the blood vessels which could explain the hemorrhagic tendency were observed. Hemorrhage was, however, easily produced prior to death by surgical incision or by trauma. In October 1941, Bingham, Meyer and Pohle[106] reported the results of similar studies on twenty-five dogs which had received hemorrhagic doses of the synthetic dicoumarin. Their observations revealed that hemorrhage had occurred in the subcutaneous and intermuscular fasciae in all the animals and that gross gastrointestinal hemorrhage and bleeding into the pleural spaces and pulmonary parenchyma were frequently observed. Marked dilatation of capillaries, small arteries and veins was also noted. No lesions of the vessel walls were found which could explain the mechanism whereby the blood was extravasated. Another interesting observation made by these authors was the absence of significant gross or microscopic evidence of parenchymatous disease of the liver, kidney or other organs. Occasional moderate hydropic degeneration of the liver was observed, but nothing more. Hydropic degeneration is known to occur post mortem, depending upon the time which has elapsed between death and tissue fixation. Roderick had previously reported liver damage as an occasional pathologic finding

in cattle which died from this disease.

Studies on the response to 3, 3′ methylene-bis-(4-hydroxycoumarin) administered orally to human subjects have been reported by Butt, Allen and Bollman,[107] Bingham, Meyer and Pohle[106] and the authors. Bingham[106] and his co-workers have also used the disodium salt of the dicoumarin intravenously. Characteristic prolongation of the prothrombin and coagulation times, similar to those observed in animals, have been noted, by all workers. The present report on the effects of the dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin) includes a description of the hemorrhagic complications which were encountered in our series in man, but which neither Bingham et al[106] nor Butt et al[107] have previously mentioned. An attempt has been made, therefore, to ascertain the nature of this variation in response.

Our objectives have been to study the reactions produced in man by this substance, the mechanism involved therein and to determine the minimum effective dose required in humans to produce an effect on the coagulation time comparable to that of heparin. In this the prothrombin reduction plays an important role. To date this dicoumarin has been administered to thirty-one patients. Detailed studies regarding twenty of these patients have been analyzed and embodied in this report. The age of the patients varied from 22 to 83 years. The distribution according to admission diagnosis is as follows:

Thromboangiitis obliterans	2
Rheumatic cardiovascular disease	2
Thrombophlebitis	4
Arteriosclerosis obliterans	12

Prior to administration of the medication, a thorough history was taken and a complete physical examination done in order to eliminate preëxisting hemorrhagic diathesis, and to avoid the possibility of causing uncontrollable bleeding from peptic ulcers, fibromyomata or other hitherto quiescent foci of potential hemorrhage.

Blood counts, including red and white cell counts with differential and hemoglobin determinations, were done on all cases before administration of the drug and at weekly intervals thereafter until prothrombin time returned to normal. Because of the wide variations obtained in platelet determinations during the control period and the feeling among hematologists that most platelet counts using available techniques are at best inaccurate, this procedure was discontinued after a trial.

Capillary fragility tests, using the method described by Wright and Lilienfeld,[108] were performed prior to administration of the dicoumarin, during the course of therapy, when prothrombin time exceeded 35 seconds, and when and if hemorrhagic manifestations appeared.

Sedimentation rates were determined by the Westergren[110] method prior to administration of the drug and at weekly intervals thereafter.

Routine urinalysis was performed three times weekly during the control period and during administration of the dicoumarin.

An attempt to estimate renal status was made on the basis of urea clearance tests, blood urea and non-protein nitrogen ratio and renal concentration tests (2-hour method described by Mosenthal).[117] These were done prior to administration of the medication with the object of reducing the possibility of retention toxicity, such as that encountered at times with other types of chemotherapy.

An evaluation of the *hepatic status* was made on the basis of the bromsulfalein test (Magath and Snell modification),[116] plasma proteins, cholesterol ester ratio and cephalin flocculation test.

Gastric fractional analysis, using parenteral histamine, was done once during the period of observation, following the suggestion of Link[109] that gastric anacidity might hasten absorption of the dicoumarin.

Prothrombin time was determined daily, using the Fullerton[111] modification of the Quick technique. Normal plasma with this method gave values of 20 seconds plus or minus 2. Daily controls performed simultaneously served as a check on the activity of the thromboplastic substance used.

Coagulation time was estimated by the Lee-White[115] two tube method with normals ranging between 5.5 and 7.5 minutes.

A three day control period preceded the administration of the drug in all cases. During this time base line coagulation and prothrombin times were ascertained.

Dosage: At the outset of our experiments no data on the dosage of this dicoumarin in humans were available. Anticipating the possibility of hemorrhagic complications, we began with doses which, on the body weight basis, were but a fraction of those which had produced prolongation of coagulation and prothrombin time in animals. Five patients received daily single, oral doses of 100 mg. for seven days followed by doses of 200 mg. at daily intervals for periods varying from four to eleven days. Of this group two patients received a total of 1500

mg. over an eleven day period; one patient a total of 1700 mg. over a twelve day period; and two a total of 2900 mg. over a period of eighteen days. Four patients received single oral doses of 200 mg. at twenty-four hour intervals for periods varying from four to eleven days. Total doses in this group were as follows: 800 mg., 1200 mg., 1800 mg., and 2200 mg.

Three patients received a series of four 300 mg. doses at twenty-four hour intervals and one patient a series of five 300 mg. doses, totaling 1200 and 1500 mg. respectively. The remaining patients received an initial dose of 600 mg. of the dicoumarin, followed in all but one case by daily doses of 200-300 mg. for periods varying from five to eight days. The amount of the dicoumarin administered to these patients total 600, 2200, 1800, 1800, 1500, 1500 and 2400 mg. The dose of the dicoumarin was administered in all instances at twenty-four hour intervals. Total dose of the body weight basis varied from 7.8 to 45.9 mg./kg.

Responses in the form of prolongation of the prothrombin and coagulation time varying widely in degree and duration were observed in patients receiving different doses and in different patients receiving the same total dose. We shall briefly outline the result of an analysis of the coagulation and prothrombin time curves obtained.

The initial effect manifested itself as a significant prolongation of prothrombin time which occurred in from one to five days, average 3.2 days. A change equivalent to three times the standard deviation for accuracy of the method was considered significant.

The maximal effect, namely, the greatest increase in prothrombin time following the first administration of dicoumarin, occurred in from one to twenty days, with an approximate average of thirteen days.

The coagulation time at the period of maximal prolongation varied from eight to thirty-three minutes, averaging thirteen minutes.

The maximal prolongation of prothrombin time ranged from twenty-six to seventy seconds, with an average of forty-seven seconds.

The total duration of effect, or time lapsing between the initial rise in the prothrombin time and its return to control level, was two to twenty-six days, averaging 11.2 days.

The duration of effect, after discontinuing the administration of dicoumarin, varied between one and twenty-three days, average 11.2 days. As a rule the degree and duration of prolongation of coagulation

TABLE I

SUMMARY OF FINDINGS ON PATIENTS IN WHOM A HEMORRHAGIC SYNDROME WAS PRODUCED BY DICOUMARIN

Case No.	Age	Total Dose		Duration of Therapy	Day on Which Hemorrhage Appeared	Proth Time During Hemorrhage	Coag Time (Lee-White) During Hemorrhagic Episode	Duration of Hemorr. Manifest.	Treatment	Signs and Symptoms
		gm.	mg/kg							
1	25	1.5g	27.2	11 days	16	54-66 sec	12-17 min	4 days	Withdrawal of Dicoumarin	(1) Gingival bleeding (2) Weakness—Vertigo
4	37	1.7g	27.7	12 days	14	34-53 sec	15-16 min	7 days	(1) Transfusion T̈ (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Spontaneous hemorrhage from wound (2) Weakness (3) Gingival hemorrhage, sublingual ecchymosis (4) Hematuria (5) Subconjunctival hemorrhage (6) Hematemesis (7) Purpura
5	45	2.9g	36.4	18 days	19	40-70 sec	16-33 min	5 days	(1) Transfusion T̈ (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Spontaneous bleeding from wound (2) Weakness (3) Purpura over trunk and extremities (4) Hematuria (5) Pharyngeal & Sublingual ecchymoses (6) Subconjunctival & Gingival hemorrhage
6	60	2.2g	38.1	11 days	12	34-48 sec	14-18 min	8 days	Withdrawal of Dicoumarin	(1) Spontaneous bleeding from wound (2) Purpura over trunk and extremities (3) Weakness and lassitude (4) Microscopic hematuria
9	60	1.2g	18.6	6 days	12	48-56 sec	12.5-22 min	10 days	(1) Transfusion T⃛ (2) Vitamin K.C.P. (3) Nicotinic Acid (4) Supportive	(1) Hematuria (2) Hematemesis (3) Sublingual ecchymosis (4) Epistaxis (5) Gingival hemorrhage (6) Weakness
10	67	1.2g	15.5	4 days	12	40-42 sec	11.5-13.5 min	5 days	(1) Vitamin K.C.P. (2) Nicotinic Acid (3) Supportive	(1) Subconjunctival hemorrhage (2) Weakness—Vertigo (3) Epistaxis
16	69	1.8g	28.5	5 days	16	38.5-53.5 sec	22-26 min	4 days	(1) Transfusion Ṫ (2) Vitamin K.C.P. (3) Nicotinic Acid	(1) Syncope (2) Hematemesis (3) Purpura over trunk & extremities
19	54	1.5g	23.6	5 days	5	38-40 sec	10-17 min	4 days	(1) Transfusion T̈ (2) Vitamin C.P. (3) Nicotinic Acid	(1) Hematemesis (2) Weakness

and prothrombin time varied directly with the magnitude of the dose and the period of time over which it was administered.

Complications: Various manifestations of a hemorrhagic tendency made their appearance in eight of the twenty patients in this series. They were as follows: Purpura 4; sublingual ecchymosis 3; conjunctival hemorrhage 3; gingival hemorrhage 4; epistaxis 2; hematuria 3; spontaneous bleeding at wound site 3; vertigo 2; hematemesis 4; weakness and lassitude 7. There were variations in extent, intensity and duration of these signs and symptoms.

Treatment of the hemorrhagic tendency: Two patients received only supportive therapy, in order to evaluate the efficacy of the various therapeutic agents employed. Five patients received daily doses of 6.4 to 12.8 mg. of 2 methyl 1, -4 naphthohydroquinone 3 sodium sulfonate, and 300 mg. of cevitamic acid parenterally in addition to 150 mg. of nicotinic acid and the grated peel of four oranges daily by the oral route. One patient received only cevitamic acid, nicotinic acid and vitamin P in the above doses. Five patients received transfusions of 250-500 cc. of whole citrated blood.

Effect of vitamins K, C, P and Nicotinic acid.[112] Synthetic vitamin K substitute in doses substantially greater than those proven to be therapeutically effective in hypoprothrombinemias due to other causes, were ineffective in lowering the prothrombin time or controlling hemorrhage produced by the administration of the dicoumarin. A further report on the effect of vitamin K substitutes on the hypoprothrombinemia induced by administration of a 3, 3′ methylene-bis-(4-hydroxycoumarin) will be embodied in a paper now in preparation. Vitamins C and P and nicotinic acid likewise exerted no detectable influence on the hemorrhagic tendency.

Effect of Transfusion. In patients showing hemorrhagic complications transfusion produced varying results. In two instances four day old bank blood was found to be ineffectual in the control of hemorrhage. Transfusion with fresh whole blood resulted in a fall in prothrombin time to a point below the hemorrhagic level, with complete cessation of hemorrhage in one case. Four other patients with hemorrhagic complications showed a temporary fall in prothrombin time with diminution in the severity of hemorrhages following transfusion. In these cases there was a subsequent prolongation of prothrombin time with recurrence of spontaneous hemorrhage requiring repetition of transfusion before control conditions were restored (cf. Table I).

TABLE II

INDICATING LACK OF RELATIONSHIP OF AGE, WEIGHT AND DOSE OF DICOUMARIN TO HEMORRHAGIC COMPLICATIONS IN 20 PATIENTS

Hemorrhagic Group						Non-Hemorrhagic Group					
Case No.	Age yr.	Body Wt. Kg	Total Dose		Duration of Therapy Days	Case No.	Age yr.	Body Wt. Kg	Total Dose		Duration of Therapy Days
			gm.	mg/kg					gm.	mg/kg	
1	25	55.0	1.5	27.2	11	2	53	73.6	2.9	39.4	18
4	37	61.3	1.7	27.7	12	3	43	85.9	1.5	17.4	9
5	45	79.5	2.9	36.4	18	7	69	51.8	1.8	34.7	9
6	60	57.7	2.2	38.1	11	8	70	68.6	.8	11.6	4
9	60	64.5	1.2	18.6	6	11	68	75.4	1.2	15.9	4
10	67	77.2	1.2	15.5	4	12	63	76 3	.6	7.8	1
16	69	63.1	1.8	28.5	5	13	68	74.5	2 2	29.5	10
18	54	63.5	1.5	23.5	5	14	48	83.1	1.5	18.0	5
						15	50	55.4	1.2	21.6	4
						17	72	56.8	1.8	31.7	5
						19	22	52.2	2.4	45.9	8
						20	82	46.3	1.5	32.3	5

DISCUSSION

Dose: There was no apparent correlation between the incidence of hemorrhagic response and the amount of the drug administered (cf., Table II); moreover, comparable degrees of prothrombin- and coagulation-time prolongation were obtained in both bleeding and non-bleeding patients. As would be expected, the period over which the prothrombin time was prolonged was greater in those patients who received the larger doses. Hemorrhagic complications of equal intensity, equally resistant to therapy were noted in patient No. 5 (Wt. 79.5 Kg.) who had received 36.4 mg./Kg. of the dicoumarin and in patient No. 9 (Wt. 64.5 Kg.) who had received only 18.6 mg./Kg.

In patient No. 5, the bleeding tendency was of five days duration, and responded to two transfusions. In patient No. 9, hemorrhagic signs and symptoms persisted over a ten day period, requiring three transfusions for adequate control.

Link[109] and his associates have observed that response to dicoumarin in animals is enhanced by malnutrition, hepatic disorders, anacidity, dehydration, and abnormally high environmental temperature. Bearing in mind these factors, as possible contributory causes to the development of the hemorrhagic diathesis, a careful analysis of available laboratory and clinical data was made which yielded the following observations:

Temperature: During the course of our investigations the room temperature on the hospital wards was at times uncomfortably high, ranging from 80°-90°, although the average temperature during this period as recorded by the U.S. Weather Bureau was 74.7° F. Whether skin capillary dilatation associated with exposure to high environmental temperature, superadded to the widespread capillary relaxation noted by Bingham,[106] was a factor in the production of hemorrhagic manifestations has not yet been ascertained.

Dehydration:[113-114] No evidence of dehydration was present in any of these patients prior to the onset of hemorrhage. Hematocrit, serum proteins, and plasma specific gravities were within normal range.

Anacidity: Gastric analysis in the patients who manifested the hemorrhagic diathesis revealed normal HCl concentration curves in four; hyperchlorhydria in one; and hypochlorhydria in three. Similar studies in non-bleeding patients showed normal HCl concentration curves in six, hypochlorhydria in three, and hyperchlorhydria in two patients.

Age: The age distribution of those patients, with and without hemorrhagic complications, was essentially the same (cf. Table II). Only three female patients were included in the series, hence no deductions regarding the influence of sex were warranted.

Nutritional status as estimated by weight and height tables was good in all but three patients. None of these showed hemorrhagic tendencies.

Capillary fragility: No apparent relationship between hemorrhagic tendency and capillary permeability could be established on the basis of the test used. There was no increase in capillary permeability over control determinations at the time of hemorrhage. Seven of the twenty patients showed abnormal capillary fragility prior to the administration of the dicoumarin. Of this group only two developed hemorrhagic complications which were not associated with a further increase in capillary fragility.

Sedimentation rate remained at control level throughout the period

of investigation in the non-hemorrhagic group. In those patients showing the hemorrhagic tendency an elevation of sedimentation rate occurred with the onset of hemorrhage, persisting until the hemorrhagic tendency subsided, at which time a slow return to control level was observed.

Renal function: Because of the inability of many of our patients to coöperate, studies of renal function by means of the urea clearance test were incomplete. Mosenthal[117] renal concentration tests showed low fixed specific gravity in four patients of the hemorrhagic group, as contrasted with one patient in the group who showed no complications.

Hepatic function: Two patients showed significant bromsulphalein retention after 1 hour. One of these (patient No. 19) showed hemorrhagic signs; the other (patient No. 11) did not. The cephalin flocculation test was positive in two non-hemorrhagic patients and in one patient who exhibited the bleeding tendency.

From the foregoing it is difficult to attribute the hemorrhagic complications observed in this group of patients to any single factor.

The interval of twenty-four to seventy-two hours which transpires before the initial effect of the dicoumarin is observed would seem to suggest that a comparable interval is required for the dicoumarin to be converted into a prothrombin inhibitor, or to exert a direct effect upon the prothrombin-producing function of the liver. Bingham, Meyer and Pohle[106] reported a similar delayed action with intravenous administration. This demonstrates that the lag is not due to retarded absorption. It cannot be stated at this time whether the site of action of dicoumarin is in the liver or not.

The fact that in vitro studies have shown that the dicoumarin, as such, has no anti-coagulant activity would tend to minimize the importance of renal retention as a probable cause of the hemorrhagic diathesis. Undoubtedly, dose-level influences the tendency to hemorrhagic complications, since other observers who administered smaller or only single doses to patients reported no such manifestations. However, in addition to this, a combination of factors which for want of adequate evidence must be termed individual susceptibility, varying from patient to patient, would seem to be an important determinant in the production of the hemorrhagic tendency.

The conclusions drawn from this study are as follows:

1. In man, the oral administration of dicoumarin will prolong proth-

TABLE III

CONTRASTING CHARACTERISTICS OF DICOUMARIN AND HEPARIN

	Dicoumarin	*Heparin*
Chemical Classification	3, 3'-Methylene-Bis-(4-Hydroxycoumarin)	Mucoitin polysulfuric acid
Preparation	1. Extraction from spoiled sweet clover hay 2. Synthesis	Extraction from blood, liver, lung and other tissues Commercially it is prepared from lung and liver
Effect Manifested by	1. Prolongation of prothrombin and coagulation times	1. Inhibition of platelet agglutination 2. Prolongation of coagulation time
Effect Antagonized by	1. Fresh whole blood	1. Trypsin 2. Thrombokinase 3. Snake venom 4. Salmine sulfuric acid
Dose	Variable (approximately 300 mg. on alternate days in recent cases has proved satisfactory)	Variable (approximately 20 mg./Kg.)
Method of Administration	1. Oral 2. Intravenous	Intravenous {Continuous Drip {Multiple Divided Dose
Initial Response Occurs	24-72 hours	Immediately
Duration of Effect	2-26 days average 11.2 days	1-4 hours
Hemorrhagic Manifestations	Purpura, Ecchymoses, Hematuria, Gingival hemorrhage, Epistaxis, Conjunctival hemorrhage	Hematomata, Hematuria, Cerebral hemorrhage
Hemorrhagic Tendency Controlled by	Transfusions of fresh whole blood or plasma	1. Salmine sulfuric acid 2. Blood transfusion

rombin and coagulation time.

2. Wide variations in degree and extent of response were observed in different patients.

3. Hemorrhagic reactions, resembling sweet clover disease in animals, occurred following the oral administration of dicoumarin in eight patients.

4. Evidence suggests that differences in individual susceptibility may be responsible in part for these hemorrhagic responses.

The contrasting characteristics of these two in vivo anti-coagulants —heparin and dicoumarin—are presented in Table III.

An attempt has been made to summarize the present status of the use of in-vivo anti-coagulants. Of these, heparin and the dicoumarin 3, 3′ methylene-bis-(4-hydroxycoumarin) appear to be the most important and the most promising. They should be considered merely as steps toward our understanding of the factors and mechanisms involved in the important process of the coagulation of the blood. Some therapeutic indications have been outlined.

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STUDIES ON EXPERIMENTAL HYPERTENSION

XVIII. *Experimental Observations on the Humoral Mechanism of Hypertension**

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THE finding that persistent hypertension may be induced in the dog by constriction of both main renal arteries or by constriction of one main renal artery and extirpation of the contralateral kidney[1] has been fully confirmed by many investigators.[2-8] Hypertension has now been produced by the same method in the monkey,[9] rat,[10-13] rabbit,[14,15] goat and sheep.[16] The development of this method was based upon the assumption that in the most common type of human hypertension in which fairly widespread vascular disease is almost an invariable accompaniment, the arterio- and arteriolosclerosis of the kidney is the primary condition which, in some way, determines the elevation of the blood pressure. Whatever may be the nature of the actual disturbance of hemodynamics which occurs in the human kidney the seat of arterio- and arteriolosclerosis, a similar effect on intrarenal hemodynamics is probably produced in animals by persistent constriction of the main renal artery. The resultant experimental hypertension is strong indication that in man too, the elevation of the blood pressure may be due to the renal vascular disease or any other kind of renal disease which might produce a similar effect on the renal circulation.

The experimental production of a disease process in animals is of value to human medicine in direct proportion to the resultant contribution to the elucidation of the cause and possible cure of its human counterpart. The various studies of experimental renal hypertension produced by constriction of the main renal artery have already brought out

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TABLE I

Subject	*Human Essential Hypertension*	*Experimental Renal Hypertension*
Cardiac rate	Normal	Normal
Cardiac output	Normal [17-19]	Normal [20]
Blood volume	Normal [21, 22]	Normal [23, 24]
Viscosity	Normal [25]	Normal [26]
Peripheral blood flow	Normal [27, 28*]	Normal [29]
Sympathectomy	Does not abolish hypertension [27]	Does not prevent or abolish hypertension [7, 23, 62, 63]
Resection of splanchnic nerves	Does not abolish hypertension [30]	Does not prevent or abolish hypertension [31]
Renal blood flow	Apparently reduced [32, 33]	Reduced [34**]
Renal excretory function	(a) Benign phase normal [35, 36]	Benign phase normal [1, 3, 5, 6, 24, 37]
	(b) Malignant phase reduced [53]	Malignant phase reduced [39]
Cardiac hypertrophy	Left ventricle when uncomplicated by failure [40]	Left ventricle. Rat,[41, 42] Rabbit,[15] Dog [3, 24]
Pulmonary arterial pressure	Not altered when hypertension is uncomplicated by left heart failure, as indicated by normal right heart [36]	Unaltered [43]
Unilateral renal disease associated with hypertension	Cured by nephrectomy when proved unilateral [44]	Cured by nephrectomy [1, 5, 6, 45]
Bilateral nephrectomy	No rise of pressure [46]	No rise of pressure [7, 47-50]
Thyroidectomy	Does not prevent or cure hypertension unless of the type associated with disease of the thyroid [51]	Does not prevent or abolish hypertension [52]
Generalized arteriolar necrosis and necrotizing arteriolitis	In malignant phase only [38, 53, 54]	In malignant phase only [55-57]

* Controversial: Abramson, D. I. and Fierst, S. M.: Resting blood flow and peripheral vascular responses in hypertensive subjects, *Am. Heart J.*, 1942, *23*:84.

** Controversial: Corcoran, A. C. and Page, I. H.: Renal aspects of experimental and clinical hypertension, *J. Lab. & Clin. Med.*, 1941, *26*:1713.

clearly the many similarities of this type to human so-called "essential" hypertension which is so frequently associated with vascular disease, especially involving the kidneys. These similarities are briefly summarized in Table I.

The presence of fairly widespread arteriolosclerosis in cases of persistent benign human hypertension and the absence of this type of change in the arterioles of dogs in the benign phase of persistent experimental renal hypertension for as long as six years, as well as the finding of medial hypertrophy in the small arteries and arterioles in both human and experimental benign hypertension, merely emphasize the probability that in man the arteriolosclerosis precedes and is not caused by the hypertension. Thus, there is adequate indication that experimental renal hypertension and human 'essential hypertension' are closely similar, if not identical, and that results obtained in studies of pathogenesis, prevention or cure of the one may be directly applied to the other.

Much evidence has now accumulated which, both directly and indirectly, shows that the elevation of blood pressure which follows constriction of the main renal arteries is due to a humoral mechanism of renal origin. It has been shown, for example, that renal denervation,[2,4,6,7,58] bilateral supradiaphragmatic excision of splanchnic nerves and lower four thoracic sympathetic ganglia,[31] subdiaphragmatic splanchicectomy with excision of celiac and upper lumbar ganglia,[6] bilateral section of anterior nerve roots from 6th dorsal to 2nd lumbar inclusive,[59] destruction of the spinal cord[60,61] and complete sympathectomy, including denervation of heart,[7,23,62,63] neither prevent nor abolish the hypertension which results from constriction of the main renal arteries. The results of these studies eliminate a nervous reflex from the kidneys as the cause of the hypertension and indicate the probable implication of a humoral mechanism.

One of the direct indications that a humoral mechanism is involved in the pathogenesis of experimental renal hypertension is the finding[64] that if the renal veins are obstructed at the same time that the renal arteries are constricted, no rise of blood pressure occurs. Another piece of evidence is the finding that when a kidney is transplanted to the neck[6] or groin,[65,66] with no nervous connections with the rest of the body, a rise of blood pressure still occurs when the main artery to the kidney is constricted. Similarly, the demonstration,[67-69] that transplantation of an ischemic kidney from one dog to the neck of a nephrectom-

ized recipient causes an immediate and sustained rise of blood pressure as soon as blood is allowed to flow through the anastomosed vessels, indicates that some substance is washed from the kidney into the systemic circulation. Finally, the actual demonstration that the blood from a kidney with its main artery constricted is actively vasoconstrictor and pressor, whether this constriction is acute or chronic, is proof of the existence of a humoral mechanism.[7,8,70-78]

That renal excretory function remains normal in benign experimental renal hypertension[1,3,5,24] indicates that retention of nitrogenous metabolites is not a causative factor. The observation that bilateral nephrectomy, which results in azotemia, causes no elevation of blood pressure[7,48-50] is additional proof that retention of nitrogenous metabolites plays no part in the pathogenesis of the hypertension but indicates clearly that the presence of the kidneys in the body is a necessary condition for the elevation of the blood pressure.

The Humoral Mechanism

The early studies on the pathogenesis of experimental renal hypertension led directly to a renewal of interest in some old observations made in 1898 by Tigerstedt and Bergman[79] who found that a saline extract of rabbit kidneys had a prolonged pressor effect when injected into another rabbit. They named the active ingredient of the renal extract "Renin." In recent years, especially since the production of experimental renal hypertension, all of the findings of Tigerstedt and Bergman have been confirmed and extended by a large number of investigations which have dealt with the preparation, purification, properties and mode of action of the renal extract which is capable of producing an elevation of blood pressure when it is injected intravenously and for which the term renin has been generally adopted.[50,80-105]

Renin:

Tigerstedt and Bergman found that renin was a protein, not dialyzable and destroyed by heat. They further found that it had a greater and more prolonged pressor effect when injected into a bilaterally nephrectomized animal, and that when renin was injected repeatedly, with intervals of only a few minutes between injections, it elicited decreasing responses of the blood pressure and eventually no response. This pheno-

menon they referred to as tachyphylaxis. Since they found that section of the spinal cord did not affect the pressor response to an intravenous injection of renin, and that renin had no effect on the isolated perfused heart, they concluded that its effect was exerted directly on the peripheral blood vessels.

In the more recent investigations these findings of Tigerstedt and Bergman have been confirmed. The results of further studies on the behavior of renin when it is subjected to ammonium sulfate precipitation and dialysis[85,94,96] suggest that it is a pseudoglobulin, but this cannot be accepted as final until it has been purified and studied by the method of electrodialysis. It has also been found[105] that in an electric field renin migrates to the cathode and that the isoelectric point of renin lies between a pH of 6.5 to 7.5.

Renin has not yet been isolated in pure form but various methods of extraction and purification have been published,[87,91,94,96,101,104] and it has been shown that the purest product so far produced is different from the known sympathicomimetic amines in that its action is not potentiated by cocaine[92] or reversed by ergotamine[93,94,96] or by piperidomethyl-3-benzodioxane.[106,107] It has also been shown that the action of renin differs from that of adrenalin, pituitrin, and tyramine in that the pressor response to an intravenous injection of renin is not associated with a decrease in peripheral blood flow or fall of skin temperature.[86,97]

Out of the early recent studies of the physiological properties of renin came the discovery that renin is not effective unless injected intravenously and that it has no vasoconstrictor properties when added to Ringer's solution which is being perfused through an isolated organ. It is, therefore, not directly a pressor substance. This was a stumbling block to the proper understanding of the mechanism of the pressor effect of renin when injected intravenously. A study by Friedman, Abramson and Marx[92] indicated the answer to this problem, for they found that when Tyrode's solution was used for the perfusion of the isolated dog's tail no vasoconstriction occurred when renin was added, but that when blood plasma was used as the perfusing fluid the addition of renin did produce marked vasoconstriction. This observation was followed by the independent and coincidental finding by Page and collaborators[108,109] and by a group of South American workers[99,100,110] that the interaction of renin and blood plasma or serum in

vitro results in the formation of a new substance which is an effective vasoconstrictor and therefore a pressor substance. Page and collaborators gave the name "renin-activator" to the substance in the blood which interacts with renin and called the resultant vasoconstrictor and pressor substance "angiotonin".[111] The South American workers named the substance in the blood "hypertensin precursor" and the vasoconstrictor and pressor substance "hypertensin".[99]

It is of interest to note here some observations first reported by Dicker[112] and confirmed by Taquini,[113,114] that when the main renal artery or the entire renal pedicle was occluded for varying periods of time, there was a prompt and prolonged rise of arterial blood pressure after the restoration of the renal circulation by release of the renal artery or renal pedicle. This contribution has been fully corroborated by other investigators.[115-119] Taquini[113] also showed that if such a completely ischemic dog's kidney was removed and the clamp on the pedicle not released until the renal vessels were anastomosed to the carotid artery and jugular vein of a nephrectomized recipient, there was a marked rise in the blood pressure of the recipient when the circulation in the completely ischemic kidney was reëstablished. This same effect was observed from the same procedure with partially ischemic kidneys from dogs with chronic renal hypertension. The result indicated that a humoral mechanism, probably identical, was involved in both cases. The study of acute, complete renal ischemia was carried further by Prinzmetal and collaborators[107,118] who demonstrated that renin was the agent present in the saline perfusates of such kidneys, and that the rise in systemic blood pressure which followed the release of the pedicle was therefore due to the liberation of renin into the general circulation. These findings have been recently confirmed.[120,121] Taquini and Braun-Menendez[120] even demonstrated renin in the systemic arterial blood of animals following release of the pedicle of completely ischemic kidneys.

The discovery that renin is not by itself a vasoconstrictor substance and the subsequent finding that it needs a substrate in the blood stream with which to produce a pressor substance has thrown light on the hitherto obscure phenomenon of tachyphylaxis. Page and co-workers[93] showed that the reason for the progressive decrease in the response to consecutive injections of renin was due to exhaustion of renin-activator in the blood. This was confirmed by the South Americans[99] who found in addition that there is an excess of renin circulating in the blood stream

of tachyphylactic animals which, along with the absence of hypertensin precursor adequately explains the reason for failure to respond to further injections of the same substance.

It has also been shown that the action of renin is not affected by hypophysectomy, thyroidectomy, pancreatectomy, gonadectomy, splenectomy, abdominal evisceration, or destruction of the spinal cord.[89,92] It is known, however, that bilateral adrenalectomy is followed by progressive decrease in the response to injections of renin.[122-124] The full significance of this latter finding is not yet completely elucidated. It will be referred to briefly again later in this paper.

It was first noted by Tigerstedt and Bergman that in bilaterally nephrectomized animals renin has a more prolonged and striking pressor effect and that tachyphylaxis is less pronounced in such animals. This has been explained by Page[109] and confirmed by the South American investigators[99] as due to an increase in the amount of renin-activator circulating in the blood stream of such "arenal" animals.

The indications that renin may be the agent which initiates the rise of arterial blood pressure following constriction of the main renal artery are further strengthened by correlating the actual effects of intravenously injected renin on various functions under various conditions with similar functions and conditions in animals with experimental renal hypertension. These are compared in Table II which shows that the physiological effects of renin, when injected intravenously into a normal animal, are identical with the observed hemodynamic state of animals hypertensive due to clamping of the main renal artery.

Even more direct evidence as to the role of renin in initiating the rise of blood pressure which follows constriction of the renal arteries is to be found in the demonstration that extracts of kidneys of animals with chronic renal hypertension[139,140] and of kidneys with acute complete ischemia[118] contain more renin than extracts of the opposite normal kidneys. Direct proof, however, that renin is concerned in the genesis of experimental renal hypertension is furnished by the demonstration of renin in renal vein blood and systemic blood of animals made hypertensive by constriction of the renal arteries.[75,76,141]

Recent studies show that renin plays a part in the maintenance of blood pressure after severe hemorrhage and in shock.[141-146] Renin is liberated from the kidneys and can be demonstrated not only in renal vein blood but also in systemic blood.[141] The liberation of the renin in these

TABLE II

Organ or Condition Studied	*Effect of Intravenous Injection of Renin in Normal Animals*	*Parallel in Experimental Renal Hypertension*
Heart Rate	Unaltered from normal.[79, 92, 98]	Unaltered from normal.[20]
Cardiac Output	Unaltered from normal [88, 125]	Unaltered from normal.[20]
Complete Sympathectomy	Does not reduce B.P. rise due to renin.[126]	Does not reduce B.P. rise in experimental renal hypertension.[7, 23, 62, 63]
Pithing	Does not reduce B.P. rise due to renin.[79, 92, 93]	Does not abolish experimental renal hypertension.[60, 61]
Hypophysectomy	Does not reduce B.P. rise due to renin.[100, 122, 127]	Does not prevent or abolish experimental renal hypertension.[128, 129]
Thyroidectomy	Does not reduce B.P. rise due to renin.[127]	Does not prevent or abolish experimental renal hypertension.[52]
Gonadectomy	Does not reduce B.P. rise due to renin.[122]	Does not prevent or abolish experimental renal hypertension.[130]
Acute Adrenalectomy	Does not reduce B.P. rise due to renin.[93]	Does not reduce rise of B.P. when ischemic kidney of hypertensive dog is grafted into neck of normal dog.[131]
Chronic Adrenalectomy	Abolishes B.P. response to renin.[122, 123, 124]	Prevents or abolishes experimental renal hypertension.[1, 6, 39, 130, 132]
	Response restored by adrenal cortical extract or D.C.S.[124]	Hypertension maintained in adrenalectomized dogs treated with adrenal cortical extract or D.C.S.[39]
Bilateral Nephrectomy	Response to renin greater.[79, 127, 133, 134, 135]	Response to grafting of ischemic kidney of hypertensive dog greater in nephrectomized than normal recipient.[8]
Peripheral Blood flow	No decrease during rise of B.P. due to renin.[86]	No decrease in hypertensive rabbits with renal ischemia.[29]
Renal Hemodynamics	Direct evidence shows decrease of renal blood flow.[89, 92, 136]	Direct evidence shows decrease of renal blood flow.[34]
	Indirect evidence of efferent arteriolar constriction.[89]	Indirect evidence of efferent arteriolar constriction.[159]
Blood Pressure	Infusion of renin causes persistent elevation.[137]	Constriction of renal arteries causes persistent elevation.[1]
	Rise not reversed by 933F.[106, 107]	The rise of B.P. due to release of the pedicle of a completely ischemic cat kidney is not reversed by 933F.[107, 118]
	Repeated injections cause tachyphylaxis.[79, 92, 93, 99]	If a cat is first rendered tachyphylactic to renin, release of pedicle of completely ischemic kidney is not followed by usual rise of B.P.[118, 121]
	Renin causes no rise in pulmonary arterial pressure.[138]	No rise of pulmonary arterial pressure in experimental renal hypertension.[43]

D.C.S. = Desoxycorticosterone acetate.
B.P. = Blood pressure.

circumstances is presumably due to the hemodynamic disturbance which is caused by the fall of blood pressure and which simulates that caused by constriction of the renal arteries.

The Substrate of Renin [Renin-Activator (Preangiotonin) or Hypertensin Precursor (Prehypertensin)] and the Nature of its Reaction With Renin

The substance in the blood stream upon which renin acts was named renin-activator by Page and co-workers and hypertensin precursor or hypertensinogen by Braun-Menendez and his collaborators. If it is eventually proved that the resultant product of the interaction of these two substances is the cause of elevated blood pressure, then the specific term "hypertensin" of the South Americans will be more pertinent than the non-specific term "angiotonin." However, until the substance in question is actually isolated from the systemic circulation of patients with essential hypertension and/or animals with experimental renal hypertension, it is well to continue to use both terminologies side by side, but to bring them in line we suggest the terms "preangiotonin" for renin-activator and "prehypertensin" for hypertensin precursor, which will be used in the remainder of this paper.

Both Page and co-workers and Braun-Menendez and his co-workers agree upon the identity and the properties of renin-activator (preangiotonin) or hypertensin precursor (prehypertensin). It has been found to be a protein, in all probability a pseudoglobulin rather than a euglobulin, since it is soluble in distilled water and is precipitated from blood serum between 0.34 and 0.6 saturation with ammonium sulfate, findings which have been confirmed in our laboratory by Dr. Yale J. Katz. It is heat labile, non-dialyzable and not ultrafiltrable and is present in blood serum and red blood cells.[99,109] It is undiminished following hyophysectomy[109] but we have found that it decreases and finally almost disappears from the systemic blood of untreated adrenalectomized male dogs. Adequate therapy with adrenal cortical hormones or desoxycorticosterone acetate results in a return of preangiotonin (prehypertensin) to the normal level. We have also found that it disappears from the blood as the result of destruction of the liver. Full details of these studies will be given in a forthcoming publication on the role of the adrenal glands in experimental renal hypertension. Page failed to find any effect on renin-activator (preangiotonin) as the result of bilateral adrenalectomy, but

he has recently reported that it disappears from the blood when the liver is removed or severely damaged by a hepatotoxic substance.[147]

It has been further found both by Page and collaborators, and the South American workers that renin-activator (hypertensin precursor) is increased in the serum of the bilaterally nephrectomized dog[93,99] and exhausted in renin tachyphylaxis. In addition, renin-activator has been reported as increased in experimental renal hypertension and human essential hypertension.[99,148,149]

The South Americans have found, in addition, that the reaction between renin and hypertensin precursor (prehypertensin) is rather specific in that the effect of renin on the substrate in the blood could not be reproduced by other enzymes such as pepsin, pancreatin, papain or extracts of liver or spleen. They also found that hypertensin precursor (prehypertensin) was present only in blood globulins and that the latter could not be replaced by hemoglobin, casein, milk, egg or blood albumin, or by liver, spleen, thymus, testes, lung, heart, skeletal muscle or vegetable proteins.[99]

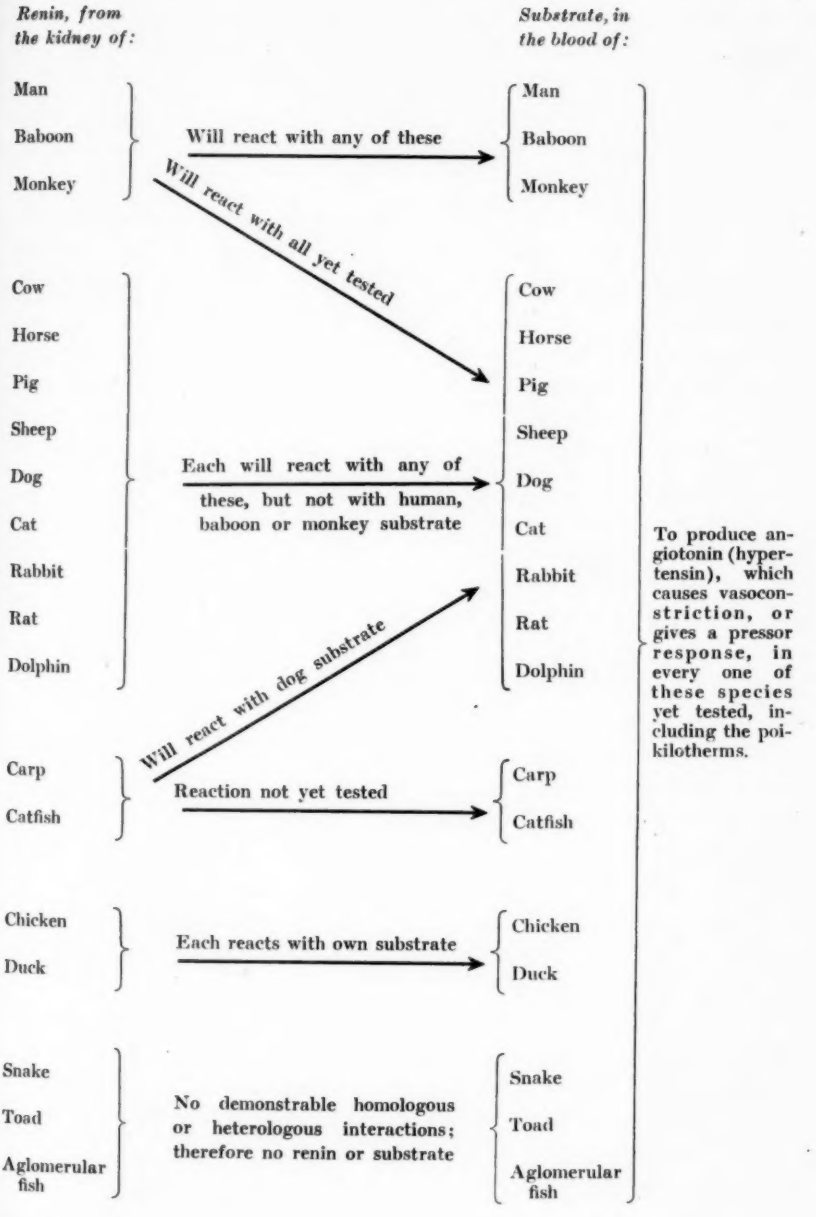
Subsequent studies with pepsin, however, by Croxatto and Croxatto[150] have shown that pepsin will produce a vasoconstrictor substance when it is allowed to act on blood globulins. They showed that the pressor substance produced by the action of pepsin was identical in its behavior to hypertensin (angiotonin) insofar as this could be chemically and pharmacologically determined. This has recently been confirmed by Helmer and Page[151] and by Dr. Yale J. Katz in our laboratory.

Interesting studies on the specificity of renin and its substrate in the blood have been made in a variety of different species by the group of South American workers[152,153] by Corcoran, Helmer and Page[154] and others.[134,155-157] These observations have been conducted on man, baboon, monkey, cow, sheep, horse, pig, dog, cat, rabbit, rat, dolphin, chicken, duck, snake, shark, toad, aglomerular fish, carp and catfish. The results of these studies are best summarized in Table III.

As to the reaction between renin and preangiotonin (prehypertensin), this seems to be enzymatic,[158] as is indicated by the following known properties of these participants and the characteristics of the reaction itself:

1. Renin is a protein, most probably a pseudoglobulin.
2. Renin is non-dialyzable and thermolabile (destroyed above 56° C[94]).

TABLE III


Renin, from the kidney of:
Substrate, in the blood of:
Man
Baboon
Monkey
Will react with any of these
Man
Baboon
Monkey
Will react with all yet tested
Cow
Horse
Pig
Sheep
Dog
Cat
Rabbit
Rat
Dolphin
Each will react with any of these, but not with human, baboon or monkey substrate
Will react with dog substrate
Carp
Catfish
Reaction not yet tested
Chicken
Duck
Each reacts with own substrate
Snake
Toad
Aglomerular fish
No demonstrable homologous or heterologous interactions; therefore no renin or substrate
To produce angiotonin (hypertensin), which causes vasoconstriction, or gives a pressor response, in every one of these species yet tested, including the poikilotherms.

3. Only a small amount of renin is necessary in proportion to the amount of blood substrate, and the amount of angiotonin (hypertensin) formed is proportional to the amount of blood globulins.
4. The reaction is affected by temperature; hastened by incubation at 37° C. and retarded or stopped at 0° C.
5. The substrate in the blood is also a protein, non-dialyzable and thermolabile.[99,109]
6. The end product of the reaction, angiotonin (hypertensin), is dialyzable and thermostabile, which indicates that the reaction is disintegrative rather than integrative, as is implied by Page's assertion that renin must be "activated."
7. The fact that renin, evidently a proteolytic enzyme, may be actually replaced by pepsin, a known proteolytic enzyme, is further proof of a disintegrative reaction. It may be that renin differs primarily from pepsin in that it acts at the natural pH of the blood and not in the pH range of pepsin activity, while pepsin cannot produce a pressor substance from blood globulins in the pH range of renin activity.

It is for these reasons that we consider renin an enzyme and preangiotonin (prehypertensin) its substrate. Page[159] agrees that this is now his working hypothesis.

Angiotonin (Hypertensin)

The substance formed by the action of renin on preangiotonin (prehypertensin) is the final effector vasoconstrictor and pressor substance of the humoral mechanism. It was independently discovered both by Page and co-workers, who named it angiotonin, and by the South American investigators, who named it hypertensin.

Page and Helmer[111] have reported the crystallization of angiotonin in the form of the picrate and oxalate, but not in uncombined pure form, and as yet have not published the melting point of any of these crystals or the probable structure of pure angiotonin. Considerable information is available, however, about the properties of this pressor substance. It is heat and acid stabile, water and alcohol soluble, fluorescent, alkali labile and gives the color reaction for arginine. Its pressor effect is immediate, the maximum rise abrupt, and the entire period of elevation of blood pressure brief, more like the pressor effect of epine-

phrine than that of renin. Renin gives a slow, maximum rise followed by a sustained elevation which may last thirty minutes or longer before returning to normal. This prolonged action of renin is assumed to be caused by the continuous liberation of angiotonin (hypertensin) due to the interaction of renin and preangiotonin (prehypertensin) in the blood stream. Page claims that angiotonin, like renin, also induces tachyphylaxis, and that "it needs its own activator", which he calls angiotonin-activator. He also found that renin destroys angiotonin when incubated with it. In addition, the pressor response to angiotonin was found to be unaffected by cocaine or atropine, or by pithing or adrenalectomy.[111] Other studies showed that the pressor response to angiotonin is greatly increased in nephrectomized dogs and that angiotonin, unlike renin, causes vasoconstriction when perfused with Ringer's solution through an isolated organ.[133] Later studies proved conclusively that the indirect effect of renin and the direct action of angiotonin are exerted on the peripheral blood vessels.[160]

The South Americans confirmed almost all of Page's findings with regard to hypertensin (angiotonin) but could not demonstrate tachyphylaxis to this substance. They also found that it produced a direct vasoconstrictor action, that its action was not reversed by piperidomethyl-3-benzodioxane (933F) and not affected by vagotomy or by excision of the carotid bodies, splanchnic nerves, liver, or adrenals, by evisceration or by destruction of the medulla.[161] They found that it was dialyzable, soluble in glacial acetic acid, liquid phenol and ethylene glycol, and that it was destroyed by pepsin.[99] Destruction by pepsin indicates that hypertensin (angiotonin) may be a polypeptide, although Page and Helmer[111] claim a negative biuret for angiotonin. Braun-Menendez and co-workers[100] also found that hypertensin was destroyed by trypsin and extracts of liver and spleen as well as by fresh normal blood serum, and that this ability of fresh serum to destroy hypertensin was decreased following bilateral nephrectomy. This substance which destroys hypertensin has been named hypertensinase by the South Americans.[99,162]

Many studies have now been reported on the physiological effects of angiotonin on the blood pressure, heart rate and output, coronary blood flow, venous pressure, viscera and renal blood flow and clearance.[163-175] In general, these correspond to the effects of the intravenous injection of renin in intact animals. Any differences that have been

found between the effects of intravenously injected renin and angiotonin may be due to the fact that the angiotonin formed from renin in vivo differs in some respects from the angiotonin produced in vitro.

The Evidence for the Existence of "Angiotonin-Activator". Page has stated,[176] "Since renin requires a second substance in order for it to exhibit pressor action, it follows that there is some reaction between these two substance to produce a third." Although Page demonstrated that renin and renin-activator form a new substance, angiotonin, yet he failed even to postulate the existence of a second new substance as the reaction product of angiotonin and angiotonin-activator.

In reference to angiotonin Page also stated,[133] "It does not appear to be the end product of the reaction between renin and renin-activator, for it itself is destroyed by further contact with renin." This statement was invalidated by the work of the South Americans,[162] who showed that another enzyme contained in renal extract, which they named hypertensinase, was responsible for the destruction of angiotonin (hypertensin) and that highly purified renin does not do it. These findings we have verified. They indicate that angiotonin (hypertensin) is the end product of the reaction between renin and renin-activator and thus further indicate that there is no need for angiotonin-activator.

In regard to the question of angiotonin tachyphylaxis, which Page observed and which he blamed partially on the exhaustion of angiotonin-activator, the South Americans[99] do not find this phenomenon in intact animals, nor do we. The South Americans and ourselves used the natural form of angiotonin produced by incubation of renin with serum. The difference in results may be due to the use of crystalline salts of angiotonin by Page and collaborators.

Again in reference to angiotonin-activator, Page said[133] "We employ the name, as in the case of renin-activator, to connote that neither renin nor angiotonin exerts a pressor action in the absence of their respective activators." It is of interest in this connection to note that angiotonin (hypertensin) will cause vasoconstriction in all species so far tested, including poikilotherms. Every species, from the kidneys of which renin has been extracted, whether this renin was effective in the same or in other species, has been found also to possess prehypertensin (preangiotonin) in its own blood. The renal extract of toad and snake,[153] prepared by the method used for the extraction of renin, is not effective

in the toad, snake or any other species. Thus the toad and snake contain no demonstrable renin or prehypertensin. Yet hypertensin (angiotonin) is effective in raising the blood pressure in both snake and toad.[153] It seems far fetched, therefore, to assume the existence of "angiotonin-activator" in animals which do not possess any of the other elements of the humoral mechanism of hypertension, yet give a definite vasoconstrictor and pressor response to the injection of angiotonin.

The difficulty of correctly ascertaining just what function Page assigns to "angiotonin-activator" or what its place in the humoral mechanism is, may well be gathered from his recent statements that "Angiotonin may be an intermediate in a series of reactions which ultimately lead to its destruction, i.e., renin + renin-activator → angiotonin + angiotonin-activator→vaso-inactive substance,"[159] and "It is my guess that angiotonin is only one in a series of substances, although probably a key substance which are formed when renin is allowed to act."[176]

It may be stated, therefore, that further evidence is needed for the existence of angiotonin-activator, and that even the demonstration of angiotonin tachyphylaxis[175] in isolated organs must be differentiated from a possible inhibitory toxic or other effect of the injected material on the blood vessels.

Hypertensinase (Angiotoninase)

Early in the course of their studies on the humoral mechanism the South Americans discovered that normal blood serum would destroy hypertensin in vitro, and they advanced the name "hypertensinase" for the responsible destructive agent. They demonstrated that hypertensinase, which was destroyed by heat (60° C.) could be separated from renin and prehypertensin, both of which are also heat labile, by incubating at 37° C. at a pH of 3.9 for fifteen minutes. Thus they showed that if the hypertensinase in renin and serum were first destroyed by this method, which leaves renin and prehypertensin unaltered, prolonged contact of renin and prehypertensin then resulted in a maximum yield of hypertensin which remained constant despite the length of incubation. The optimum pH of hypertensinase activity was found to be between 7.5 and 8.5, and its action to be affected by temperature, for at 0° C. its action stops. Thus they believe it to be an enzyme. It can be precipitated by half-saturation with ammonium sulfate, but not by dialysis.

In their study of the hypertensinase content of various tissues Fasciolo et al[162] found that kidney cortex had 1500, intestinal mucosa 1200-1600, pancreas and spleen 200, hemolyzed red cells and liver 100 units per gram. Serum and plasma (without hemolysis) were found to have only about 1 unit per gram.

That the kidney may be the main source of the hypertensinase in normal blood is indicated by the finding that there is almost a complete disappearance of hypertensinase from the blood stream of bilaterally nephrectomized dogs.[162]

Harrison, Grollman and Williams[177-179] were the first to attempt the treatment of experimental renal hypertension with renal extracts, although they had made no in vitro studies of the action of these extracts on angiotonin (hypertensin). Their approach was based on the observations that the normal kidney, by some humoral means, might play a part in eliminating the hypertensive effect of a contralateral ischemic kidney, since the blood pressure of such an animal tends to return to normal, and also that when the normal kidney is removed, in the presence of the ischemic one, the blood pressure ascends to and remains at a much higher level.

The idea that the normal kidney might play a part in the elimination of the "chemical mediator" in experimental hypertension was, incidentally, studied by Katz and Rodbard,[180-182] who confirmed the fact that the blood pressure falls to normal in six hours after the removal of a single ischemic kidney[6] and also observed that it takes five times as long for it to reach normal after removal of both kidneys when one or both were ischemic. They believed that the more rapid fall of the blood pressure to normal levels in the presence of normal kidney tissue, as contrasted with the slow fall in its absence, might be due to the excretion or in vivo destruction of the "chemical mediator" by the remaining normal kidney. By constructing a uretero-venous fistula on the side of the normal kidney they showed that removal of the opposite ischemic kidney was still followed by a rapid, six hour fall of the blood pressure to normal, and concluded that the normal renal tissue destroyed the "chemical mediator," since its excretion back into the blood stream would have kept the blood pressure elevated for a length of time comparable to that necessary in the absence of any renal tissue.[182] Since then, Dexter and Braun-Menendez[183] have demonstrated that the renal threshold of renin is too high for excretion of renin to account for the

stabilizing role of the normal kidney.

Independently of Harrison and collaborators, Page and his collaborators[184-186] reported the treatment of hypertension with renal extracts containing angiotonin-inhibitor. The basis of their approach was different from that of the South Americans and of Harrison and co-workers, for they[133] had previously published their findings that renin and angiotonin tachyphylaxis were both due to more than simple exhaustion of renin-activator and angiotonin-activator, and had postulated the presence of renin-inhibitor and angiotonin-inhibitor. They believed that it was the loss of these two inhibitors after nephrectomy that accounted in large measure for the increased sensitivity of nephrectomized animals to renin and angiotonin. Although in their first publication they claimed that the blood of hypertensive animals treated with their renal extracts no longer contained renin-activator or angiotonin-activator,[184] neither this finding nor the existence of renin-inhibitor has been confirmed. All of their work was done in vivo, but recently they[187,188] have confirmed the results of Fasciolo and collaborators[162] on the ability of extracts of various organs to destroy angiotonin in vitro, which we have also confirmed.

Since the existence of a substance which destroys hypertensin (angiotonin) was first described by the South Americans and shown to be due to an enzyme which they named hypertensinase, we believe that to keep the terminologies similar, until a final one is adopted, the corresponding term angiotoninase is apropos for "angiotonin-inhibitor," for the action of the enzyme in question is not that of inhibition of angiotonin, or suppression of renin- or angiotonin-activator, but the actual destruction of angiotonin itself. Because of the inconsistency of their results, the South Americans[141] did not publish the effect of treatment of experimental renal hypertension with renal extracts containing hypertensinase. Since it has not yet been shown that the possible antipressor effect of renal extracts of various kinds that have been used for the treatment of hypertension is actually due to hypertensinase (angiotoninase) it is well for the present to refer to such extracts as antipressor renal extracts.

Friedman[164] demonstrated that renal vein blood from an ischemic dog's kidney contained much less hypertensinase than renal vein blood from normal kidneys. This throws light on the earlier experiments by Freeman[189] who found that normal dog's blood would reduce the blood

pressure of dogs hypertensive due to constriction of the main renal arteries, whereas blood from hypertensive dogs gave no such effect. In this case the antipressor effect may have been due to the presence of hypertensinase in normal blood and its diminution in the blood of hypertensive animals, just as other previously cited results in bilaterally nephrectomized animals may have been due to the absence of hypertensinase in this condition.

Non-Specific Pressor and Anti-Pressor Substances

An indication of the part which the kidney might play in the elaboration of at least one of the substances involved in the humoral mechanism of hypertension has been given by a series of important studies by Bing and collaborators.[190-193] They have demonstrated that an ischemic kidney is capable, by effecting decarboxylation, of converting l-dopa (l-dihydroxyphenylalanine), a substance with no pressor properties, into hydroxytyramine, a powerful pressor substance. The amount of hydroxytyramine formed in the kidney from dopa, only under conditions of oxygen lack, was found to be proportional to the reduction of blood flow through the perfused kidney.[191] On the contrary, l-dopa added to normal blood being perfused through a kidney with normal blood flow was not converted into hydroxytyramine. It is of special significance that although liver and intestine also contain l-dopa-decarboxylase, yet these organs were unable, even when their circulation was reduced, to produce hydroxytyramine from dopa added to the blood. Similarly, it is only when the main renal artery is constricted that experimental hypertension results in the dog.[194,195] In further experiments on artificially perfused kidneys, it was found that the ischemic human kidney also converted dopa to hydroxytyramine.[190] The above experiments were done with kidneys in vitro, but Bing was also able to show that partially or completely ischemic cat kidneys converted dopa into hydroxytyramine in vivo.[192] As the result of these studies Bing has not concluded that hydroxytyramine is the cause of, or is in any way involved in, the pathogenesis of experimental renal hypertension, for he has demonstrated that angiotonin (hypertensin) is destroyed by a different fraction of renal extract than is hydroxytyramine, and that these two pressor substances are destroyed by different mechanisms.[193] This fact, along with his finding that renin was unable to effect decarboxylation of dopa and convert it to hydroxytyramine,[190] helps

to emphasize Bing's contention that he does not claim that hydroxytyramine is the cause of experimental renal hypertension. In this respect he has already been misquoted.[196] He has merely intended to show that when the blood flow to a kidney has been reduced the organ behaves differently than when the circulation is normal and that under these conditions it is capable of converting an amino acid, itself without pressor properties, into a powerful pressor amine.

Schroeder and Adams[197,198] have found that tyrosinase, a phenolic oxidase, has the power to destroy angiotonin in vitro. In all of their tests in which tyrosinase alone was added to angiotonin, and the two shaken together, very little oxygen was absorbed and the angiotonin lost none of its pressor activity. When a small amount of blood serum, however, was added to the mixture of tyrosinase and angiotonin, oxygen was rapidly absorbed and the angiotonin was completely inactivated. Schroeder and Adams concluded that tyrosinase destroyed the angiotonin, but in the light of our present knowledge it is only fair to conclude that the effect may have been due to the hypertensinase in the blood serum and not to the tyrosinase itself. Nevertheless, Croxatto and Croxatto[199] found that tyrosinase from mushrooms and amine oxidase from the liver of the squid, did destroy the vasoconstrictor activity of hypertensin as tested by Laewen-Trendelenberg technique on the toad.

The presumable destruction of angiotonin by tyrosinase led Schroeder[198] to postulate that angiotonin, like adrenalin and tyramine, "may accordingly contain a phenolic group." On this basis he undertook the treatment of experimental and human hypertension with tyrosinase and found that it reduced the blood pressure. Martin and collaborators[196] also found that tyrosinase will lower the blood pressure of hypertensive dogs, but added that this effect of tyrosinase was greatly enhanced by the addition of catechol.* How these results are related to the antipressor effects of renal extracts in experimental renal and in human hypertension is far from clear. When it is realized that such substances as typhoid vaccine and sterile milk[200] can also cause a reduction of blood pressure in experimental renal and human hypertension, the intricacy of this phase of the problem of hypertension becomes obvious. The present status of the treatment of experimental renal hypertension is reviewed in full in a forthcoming publication.[200]

* In a personal communication from Dr. Myron Prinzmetal[201] we have learned that a mushroom tyrosinase preparation, in which the tyrosinase was inactivated by heat, was also effective in producing a significant lowering of blood pressure and remission of other symptoms of human hypertension, when the extract was administered by intramuscular injection.

Summary

It has been demonstrated that after constriction of the main renal arteries to a sufficient degree to produce experimental renal hypertension, renin, an enzyme, is liberated into renal vein blood. It has been shown that this enzyme, renin, interacts with preangiotonin (prehypertensin) a pseudoglobulin substrate in systemic blood, probably produced by the liver in the presence of an adequate amount of functioning adrenal cortical tissue, to form the final effector vasoconstrictor and pressor substance, angiotonin (hypertensin). It has also been demonstrated that the physiological effects of the intravenous injection of renin are identical to the hemodynamic alterations found in experimental renal hypertension and in human essential hypertension. It follows, therefore, that the humoral mechanism noted above may also be responsible for the elevation of blood pressure in human essential hypertension. Although the demonstration in hypertensive blood of vasoconstrictor substances which may possibly be related to this humoral mechanism, has been reported, the proof that angiotonin is the responsible agent, awaits its isolation from the systemic blood of human beings with hypertension.

In addition to renin, preangiotonin (prehypertensin) and angiotonin (hypertensin) which are involved in the humoral mechanism of experimental renal hypertension, a fourth factor, hypertensinase (angiotoninase) has been discovered. Although this substance, an enzyme, destroys angiotonin (hypertensin) in vitro, there is no proof yet available that it is effective in lowering blood pressure in man or animals with hypertension. It has not yet been shown that the anti-pressor renal extracts with which hypertension is said to have been lowered in man and animals are rich in hypertensinase and inactive when this enzyme is destroyed. The exact nature of the effective substance in anti-pressor renal extracts which have been used in the treatment of hypertension, remains to be elucidated.

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*RECONSTITUTED MILK**

1. *Definition*

Reconstituted milk is a fluid resembling ordinary milk in appearance and taste; it is prepared from butterfat, dried skim milk and water by means of emulsifying apparatus. When prepared by exposure to temperatures of 150° F. or less, skim milk powder preserves its emulsifying qualities. Such low temperatures cannot be used in the process of drying whole milk since lipolytic enzymes are not destroyed at these temperatures and they react on the milk fats with a tendency to the development of rancidity. Temperatures above 175° F. are necessary to preserve the fat of dried whole milk in the powdered state. High temperatures coagulate the milk proteins and make them insoluble. The particles remain suspended in the water and the taste of the milk is objectionable. For this reason whole dried milk and evaporated milk are unsatisfactory as beverages. The term "reconstituted milk," therefore, as used here, refers to a palatable emulsified fluid made of butterfat, of skim milk dehydrated at a temperature of 150° F. or below, and of water.

2. *Comparison with Fresh Milk*

Analyses of the reconstituted milk supplied us by Dr. Charles E. North were made in the laboratories of two of the members of the Subcommittee. These bear out the evidence of other laboratories that the chemical and nutritional components of reconstituted milk are the same as of fresh milk except that there is a diminution of vitamin C. The pH of the milk as well as the mineral content, although varying slightly from sample to sample, appeared to correspond with those in fresh milk.

The methods of production of reconstituted milk are wholly susceptible to adjustments with regard to the amount of butterfat in the milk and as to sanitary requirements. Reconstituted milk can be pasteurized in the same way as ordinary milk. The quality of the constituents of the milk can be adequately supervised. In other words, nutritionally, chemically, and bacteriologically, and by taste, reconstituted milk can be made the equal of natural milk. In saying this the Subcommittee is not concerned with the question of whether or not reconstituted milk should be encouraged where ordinary milk is available in adequate quantities to meet the needs of the community.

3. *The Field of Usefulness of Reconstituted Milk*

The greatest field of usefulness for reconstituted milk is in areas removed from dairy cattle regions, in sub-tropical and tropical climates, and on ships and in army cantonments under war conditions. It can be used as a subsidiary supply in large communities during periods of milk shortage, and, if it be true that it can be sold at much lower prices than regular fluid milk, its availability to people of small income may become a matter of considerable importance.

By dehydrating skim milk and storing it for future use during the spring and early summer when milk is particularly abundant and rich in butterfat, the national economy is improved and wastes are eliminated. It must also be borne in mind that reconstituted milk can be made anywhere by the use of proper apparatus and thus the need of shipping water in milk is obviated.

4. *Experiments with Reconstituted Milk*

During the latter part of World War I the subject of milk dehydration and of milk reconstitution aroused great interest. Experiments in the use of reconstituted milk were carried out, including clinical tests at the Babies' Hospital in New York and elsewhere. The experiments showed that adults as well as babies can thrive on reconstituted milk made of properly safeguarded ingredients.

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* Report by Subcommittee of the Committee on Public Health Relations of The New York Academy of Medicine, March 21, 1942.

5. *Summary*

In recording its approval of reconstituted milk, the Subcommittee does not endorse any particular type. The ingredients may comprise different varieties of dehydrated skim milk and different types of butterfat, whether it be melted butterfat, or sweet butter, or plastic cream, or butter oil, the latter being preferable because of stability under practically all conditions of storage. The main considerations are that the milk be safe, nutritious and palatable.

Whether or not the claims are true that reconstituted milk of good quality can be produced at a cost considerably below that of ordinary milk is a matter on which the Subcommittee has no valid opinion to offer.

6. *Recommendations*

The Subcommittee recommends:

A. That the Academy endorse the use of reconstituted milk in those areas where natural milk of proper hygienic standard is not easily obtainable, provided that:

1. When sold, reconstituted milk be adequately labeled to distinguish it from other milks;
2. Reconstituted milk conform to the chemical and bacteriological standards required of fresh milk; and
3. The skim milk used in reconstituted milk be dehydrated at a temperature of 150° F. or less.

B. That copies of this report be transmitted to the Surgeons General of the Army, Navy, and Public Health Service; to the Departments of Health of the City of New York and the State of New York; and to the Children's Bureau of the U. S. Department of Labor.

C. That this report be submitted for publication to the Editorial Board of the Bulletin of The New York Academy of Medicine.

Respectfully submitted:

HAVEN EMERSON, *Chairman*
F. ELMER JOHNSON
SAM Z. LEVINE
A. A. WEECH
H. B. WILCOX
E. H. L. CORWIN, *Secretary*

Approved by the Committee on Public Health Relations, April 6, 1942.

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PROCEEDINGS OF ACADEMY MEETINGS

STATED MEETINGS

APRIL 2—*The New York Academy of Medicine.* Executive session—a] Reading of the minutes; b] Presentation of certificates of fellowship. The Seventeenth Hermann Michael Biggs Memorial Lecture "Nutrition and the Nation at War," James S. McLester, Professor of Medicine, University of Alabama. Report on election of Fellows and Corresponding Fellow. Report on election of Recording Secretary.

APRIL 16—*The Harvey Society (in affiliation with The New York Academy of Medicine).* The Seventh Harvey Lecture, Lymphatic Participation in Cutaneous Phenomena," Philip D. McMaster, Associate Member, The Rockefeller Institute for Medical Research.

SECTION MEETINGS

APRIL 7—*Dermatology and Syphilology.* Presentation of cases—Skin and cancer unit of Post-Graduate Hospital and Medical School, Columbia University. ¶ Discussion. ¶ Executive session — Nomination of Section Officers and one member of Advisory Committee.

APRIL 9—*Combined Meeting: Genito-Urinary Surgery and Pediatrics.* ¶ Papers of the evening—a] Wilms' tumor, Sydney Farber (by invitation); b] Indications for urological investigation in children, John D. Lyttle. ¶ Discussion, Meredith F. Campbell. ¶ Executive session—a] Reading of the minutes; b] Nomination of Section Officers and one member of Advisory Committee for each section.

APRIL 10—*Surgery.* Reading of the minutes. ¶ Presentation of cases—a] 1. Lymphosarcoma of the stomach with perforation. Gastric resection. Recovery one-year post-operative. 2. Mesentery thrombosis of the terminal ileum. Resection of ileum. Ileocecostomy. McBurney incision. Four-months post-operative, William T. Doran, Jr.; b] Cases illustrating paper of the evening, John H. Garlock, Leon Ginzburg. ¶ Paper of the evening — Complications and causes of mortality of the surgical treatment of carcinoma of the colon and rectum, John H. Garlock, Leon Ginzburg, Ameil Glass (by invitation). ¶ General discussion. ¶ Executive session — Nomination of Section Officers and one member of Advisory Committee.

APRIL 14—*Neurology and Psychiatry.* Reading of the minutes. ¶ Presentation of case — Gastric function in association with varying emotional states, as stud-

ied in a man with a large gastric fistula since 1895, Harold G. Wolff, Stewart Wolf (by invitation). ¶ Papers of the evening—a] Varieties of vertigo in acoustic neuromas and the Ménière syndrome, Earl Chesher (by invitation), discussion by Miles Atkinson (by invitation); b] Ambulatory insulin technique in the treatment of schizophrenia, Phillip Polatin (by invitation), Hyman Spotnitz, discussion by Nolan D. C. Lewis (by invitation). ¶ Executive session — Nomination of Section Officers and one member of Advisory Committee.

April 15—*Otolaryngology.* Reading of the minutes. ¶ Papers of the evening— a] Study of impairment of hearing in children, LeRoy M. Polvogt, Johns Hopkins University (by invitation), Discussion, Edmund P. Fowler; b] Treatment of battle casualties in the Navy, Captain Reynolds Hayden, M.C., U.S.N., Former Commanding Officer, Naval Hospital, Pearl Harbor (by invitation). ¶ General discussion. ¶Executive session —Nomination of Section Officers and one member of Advisory Committee.

April 17—*Orthopedic Surgery.* Executive session—a] Reading of the minutes; b] Nomination of Section Officers and one member of Advisory Committee. ¶ Presentation of cases—Cases illustrating arthroplasty for congenital dislocation of the hip, Paul C. Colonna. ¶ Papers of the evening—a] Early treatment of congenital dislocation of the hip (illustrated by x-ray slides and motion pictures), Arthur Krida; b] Reconstruction of the acetabulum in congenital dislocation of the hip; indications, technique, and end-results (illustrated by x-ray slides and motion pictures), A. Bruce Gill (by invitation); c] An arthroplasty for congenital dislocation of the hip, late follow-up report (illustrated by x-ray slides and motion pictures), Paul C. Colonna; Discussion by Lieut. Commander Francis J. Carr, Leo Mayer. ¶ General discussion.

April 20—*Ophthalmology.* ¶ Exhibit: 7:00 to 8:15 o'clock. Tumors of the eye, presented by the Army Medical Museum, Helenor C. Wilder, Washington, D. C. (by invitation). ¶ Demonstration: 7:00 to 7:45 o'clock. The reading of pupillographic curve. Otto Lowenstein and Mark J. Schoenberg. ¶ Executive session: 8:30 o'clock—a] Reading of the minutes; b] Nomination of Section Officers and one member of Advisory Committee. ¶ Presentation of cases—a] A case of tuberculosis of the conjunctiva, Olga Sitchevska (by invitation); b] A case of bilateral subluxation of lenses, needling of posterior capsule, James W. Smith; c] A case of argyrosis of the cornea, Abraham J. Lanchner (by invitation). ¶ Papers of the evening—a] Pupillographic studies in simple glaucoma, Otto Lowenstein and Mark J. Schoenberg; b] The biologic theory of refraction states versus the use-abuse theory, E. V. L. Brown, Chicago (by invitation), discussion by Willis S. Knighton.

April 21—*Medicine.* ¶ Executive session—a] Reading of the minutes; b] Nomination of Section Officers and one member of Advisory Committee. ¶ Presentation of cases (by invitation)—a] Marchiafava-Micheli syndrome, Emanual B. Schoenbach, Mount Sinai; b] Mitral stenosis complicated by embolization of the right profunda femoris artery — successful embolectomy. (Notes on the use of heparin postoperatively), James Otto, Roosevelt Hospital; c] Congestive heart failure of obscure etiology—a case of eight-years' duration, J. James Smith, New York Hospital; d] Fatal trichinosis with myocardial involvement, George A. Perera, Presbyterian Hospital; e] A case of cerebral trichinosis with recovery, Sarah Flanders, Bellevue Hospital, Second Division; f] A case of lupus erythematosus, Charles Yongue, Bellevue Hospital, First Division; g] Scleroderma — a generalized syndrome, Sydney Steinberg, Post-Graduate Hospital; h] An unusual case of tricuspid endocarditis, William H. Harris, Bellevue Hospital, Third Division;

i] A case of splenomegaly of twelve years' duration, Frank Rummel, Bellevue Hospital, Fourth Division; j] Fiedler's-isolated myocarditis, William Sicher, Lenox Hill Hospital; k] Malignant paranganglioma, Solomon D. Klotz, Metropolitan Hospital; l] Bronchogenic carcinoma in a child, Carl Bornemann, Flower Fifth Avenue Hospitals.

Genito-Urinary Surgery—The regular meeting of the Section was not held, as the Section had held a combined meeting with the Section of Pediatrics on April 9.

APRIL 28—*Obstetrics and Gynecology.* ¶ Executive session—a] Reading of the minutes; b] Nomination of Section Officers and one member of Advisory Committee; ¶ Papers of the evening*—a] The pulse and respiratory variations during labor in women with rheumatic heart disease, Harold E. B. Pardee, Curtis L. Mendelson (by invitation), discussion by Harold E. B. Pardee; b] Habitual abortion, Carl T. Javert (by invitation), discussion by Locke Muckenzie; c] Transfusion reactions, Charles E. Robinson (by invitation); d] Impetigo contagiosa of the newborn, Leston E. Fitch (by invitation). ¶ General discussion.

*Papers presented by members of staff of the Lying-In Hospital.

AFFILIATED SOCIETIES

APRIL 20—*New York Roentgen Society (in affiliation with The New York Academy of Medicine).* ¶ Presentation of cases—a] Case from Memorial Hospital, Ralph E. Herendeen; b] An undiagnosed gastric lesion, Frank Huber; c] 1. Leukemia of bone, simulating tuberculosis, 2. Neurotrophic hip joints in a boy fifteen years of age, Raymond W. Lewis; d] Xanthoma in relation to giant-cell tumor, J. H. Levy; e] An unusual neoplasm of the lung, Ernest H. Wood, Jr. (by invitation); f] Renal aneurysm, Robert J. MacCallum (by invitation); g] Polycythemia vera treated with generalized irradiation, Clara Okrainetz; h] Radiosensitivity of carcinoid, William V. Tenzel. ¶ General discussion. ¶ Executive session.

APRIL 23—*New York Pathological Society (in affiliation with The New York Academy of Medicine).* ¶ Papers of the evening: Dynamic Aspects of the Pathological Processes of Chronic Renal Lesions—a] The application by the physiologist of the titration method to the study of the distribution of glomerular filtrate and tubular perfusion, Homer Smith (by invitation); b] The recognition by the clinician of the shifting anatomical alterations in the kidney by variation in the urinary sediment, Thomas Addis (by invitation), discussion by Paul Klemperer. ¶ Executive session.

DEATHS OF FELLOWS

BROOKS, FRANK TERRY: 1833½ North Grace Avenue, Hollywood, California; born in Massachusetts, February 15, 1867; died in Los Angeles, California, January 17, 1942; received the degree of B.A. from Yale University in 1896; graduated in medicine from the Long Island College Hospital in 1893; elected a Fellow of the Academy October 4, 1900.

DELAVAN, DAVID BRYSON: 30 East 60 Street, New York City; born in New York City, May 1, 1850; died in New York City, May 23, 1942; received the degree of B.A. from Yale College in 1872; graduated in medicine from the College of Physicians and Surgeons, Columbia University, in 1875; elected a Fellow of the Academy January 8, 1880; served the Academy as Secretary of the Section on Otolaryngology (then known as the Section on Laryngology and Rhinology) during the year 1886, and as Chairman of that Section from 1894 to 1896; as Corresponding Secretary of the Academy from 1914 to 1926, when that office was abolished; and as a member of the Academy Library Committee from 1887 to 1889, and as its Chairman during the year 1889.

Dr. Delavan was at one time professor of laryngology and rhinology at the New York Polyclinic Medical School and Hospital, and consultant in laryngology and rhinology to the Mt. Sinai Hospital, the Skin and Cancer Hospital, St. Luke's Hospital, Memorial Hospital, and the Hospital for the Ruptured and Crippled (the latter hospital now known as the Hospital for Special Surgery). He was an Honorary Fellow of the American Laryngological Association, and its president in 1893 and again in 1918, a Fellow of the American Medical Association, a Fellow of the American College of Surgeons, a diplomate of the American Board of Otolaryngology, a charter member of the American Society for the Control of Cancer, and a member of the State and County Medical Societies.

DWORETZKY, JULIUS PETER: 7 Law Street, Liberty, New York; born in Lida, Russia, December 24, 1885; died in Liberty, New York, April 20, 1942; graduated in medicine from the Long Island College Hospital in 1910; elected a Fellow of the Academy April 2 1936.

Dr. Dworetzky was director of medicine at the Municipal Sanatorium at Otisville; consulting physician to the Elizabeth A. Horton Memorial Hospital at Middletown, St. Clare's Hospital in New York City, St. Francis Hospital at Port Jervis; Ulster County Tuberculosis Hospital at Kingston; and medical examiner to the Veterans Bureau. He was a Fellow of the American College of Physicians, a Fellow of the American Medical Association, a diplomate of the American Board of Internal Medicine, a diplomate of the American Board of Otolaryngology, a Fellow of the American College of Chest Physicians, and a member of the American Laryngological, Rhinological and Otological Society, and the State and County Medical Societies.

During the World War, Dr. Dworetzky served in France as a Captain of the American Red Cross with the Rockefeller Tuberculosis Commission.

Dr. Dworetzky made several original contributions to literature in the field of tuberculosis of the larynx.

TOWNSEND, TERRY MONROE: 101 East 74 Street, New York City; born in Jeffersonville, Indiana, November 2, 1876; died in New York City, May 16, 1942; graduated in medicine from the Medical Department, University of Louisville in 1897; elected a Fellow of the Academy, April 3, 1902.

Dr. Townsend was director of urology at the Lutheran Hospital and at the Department of Correction Hospital; and consulting urologist to the Midtown, Morrisania, St. Clare's and Ossining Hospitals. He was a Fellow of the American Medical Association, a Fellow of the American College of Surgeons, a diplomate of the American Board of Urology, a member of the County Medical Society and its president in 1933, and a member of the State Medical Society and its president in 1939 and 1940.